

**AMENDMENTS TO THE CLAIMS:**

This listing of claims will replace all prior versions and listings of claims in the application:

1. (Withdrawn) A sulfur atom-free enzyme protein comprising 18 types of L-amino acid residue: L-alanine; L-aspartic acid; L-glutamic acid; L-phenylalanine; L-glycine; L-histidine; L-isoleucine; L-lysine; L-leucine; L-asparagine; L-proline; L-glutamine; L-arginine; L-serine; L-threonine; L-valine; L-tyrosine; and L-tryptophan.
2. (Withdrawn) The sulfur atom-free enzyme protein according to claim 1 which retains the activity of the original enzyme protein and has oxidation resistance, wherein L-cystein and L-methionine residues in enzyme proteins comprising 20 types of L-amino acid residue: L-alanine; L-aspartic acid; L-glutamic acid; L-phenylalanine; L-glycine; L-histidine; L-isoleucine; L-lysine; L-leucine; L-asparagine; L-proline; L-glutamine; L-arginine; L-serine; L-threonine; L-valine; L-tyrosine; L-tryptophan; L-cystein; and L-methionine, are substituted with 18 types of L-amino acid residue: L-alanine; L-aspartic acid; L-glutamic acid; L-phenylalanine; L-glycine; L-histidine; L-isoleucine; L-lysine; L-leucine; L-asparagine; L-proline; L-glutamine; L-arginine; L-serine; L-threonine; L-valine; L-tyrosine; and L-tryptophan.
3. (Withdrawn) The sulfur atom-free enzyme protein according to claim 2 wherein amino acid substitution is carried out by site-directed mutagenesis using synthetic DNA.

4. (Withdrawn) The sulfur atom-free enzyme protein according to any one of claims 1 to 3 wherein the enzyme activity is oxidation-reduction activity or hydrolysis activity.

5. (Withdrawn) The sulfur atom-free enzyme protein according to any one of claims 1 to 4, which retains the activity of dihydrofolate reductase and has oxidation resistance.

6. (Withdrawn) The sulfur atom-free enzyme protein according to any one of claims 1 to 4, which retains the activity of xylanase and has oxidation resistance.

7-10 (Cancelled)

11. (New) A method of producing a sulfur atom-free enzyme protein that retains activity, wherein the sulfur atom-free enzyme protein is prepared by a combined mutation method comprising:

(1) preparing a mutant gene by substituting an initiation codon encoding L-methionine in a DNA sequence encoding an enzyme protein having an amino acid sequence comprising n number of sulfur atom-containing amino acids, wherein a position of a sulfur-containing amino acid on the sequence is  $A_i$  ( $i = 1$  to  $n$ ), with codons encoding any of L-methionine-L-alanine, L-methionine-L-serine, or L-methionine-L-proline; expressing the mutant gene in a host cell to obtain a mutant enzyme protein; measuring enzyme activity of the mutant enzyme protein; and selecting the mutant enzyme protein with the highest

activity, thereby obtaining a mutant enzyme protein having a substitution

A1/MA1;

(2) preparing a mutant gene in which a codon encoding a sulfur-containing amino acid at a position  $A_i$  ( $i = 2$  to  $n$ ) is substituted with a codon encoding any of L-alanine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-glycine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-asparagine, L-proline, L-glutamine, L-arginine, L-serine, L-threonine, L-valine, L-tyrosine, and L-tryptophan, for a maximum of 18 different substitutions at any  $A_i$ ; expressing the mutant gene in a host cell to obtain a mutant enzyme protein; measuring enzyme activity of the mutant enzyme protein; and selecting a maximum of three mutant enzyme proteins having the highest activity, thereby obtaining mutant enzyme proteins having substitutions  $A_i/B_{i1}$ ,  $A_i/B_{i2}$ , and  $A_i/B_{i3}$ , wherein activity decreases in order  $A_i/B_{i1} > A_i/B_{i2} > A_i/B_{i3}$ ;

(3) repeating (2) for all  $A_i$ ; and

(4) producing a sulfur atom-free enzyme protein comprising any combination of the substitutions in (2) and (3) with the substitution of A1/MA1 in (1), wherein a substitution occurs at all  $A_i$ ; measuring enzyme activity of the sulfur atom-free enzyme protein; and selecting a sulfur atom-free enzyme protein that retains activity.

12. (New) A method of producing a sulfur atom-free enzyme protein that retains activity, wherein the sulfur atom-free enzyme protein is prepared by a stepwise mutation method comprising:

(1) preparing a mutant gene by substituting an initiation codon encoding L-methionine in a DNA sequence encoding an enzyme protein having an amino acid sequence comprising n number of sulfur atom-containing amino acids, wherein a position of a sulfur-containing amino acid on the sequence is A<sub>i</sub> (i = 1 to n), with codons encoding any of L-methionine-L-alanine, L-methionine-L-serine, or L-methionine-L-proline; expressing the mutant gene in a host cell to obtain a mutant enzyme protein; measuring enzyme activity of the mutant enzyme protein; and selecting the mutant enzyme protein with the highest activity, thereby obtaining an A1/MA1 mutant;

(2) preparing a mutant gene in which a codon encoding a sulfur-containing amino acid at A<sub>2</sub> of the A1/MA1 mutant of (1) is substituted with a codon encoding any of L-alanine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-glycine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-asparagine, L-proline, L-glutamine, L-arginine, L-serine, L-threonine, L-valine, L-tyrosine, and L-tryptophan, for a maximum of 18 different substitutions at A<sub>2</sub>; expressing the mutant gene in a host cell to obtain a double mutant; measuring enzyme activity of the double mutant; and selecting a maximum of three double mutants with the highest activity;

(3) preparing a mutant gene in which a codon encoding a sulfur-containing amino acid at A<sub>3</sub> of the double mutants is substituted with a codon encoding any of L-alanine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-glycine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-asparagine, L-proline, L-glutamine, L-arginine, L-serine, L-threonine, L-valine, L-tyrosine, and L-

tryptophan, for a maximum of 18 different substitutions at A3; expressing the mutant gene in a host cell; measuring enzyme activity of the triple mutant; and selecting a maximum of three triple mutants with the highest activity; and

(4) repeating the stepwise substitution of codons encoding sulfur containing amino acids at each remaining A<sub>i</sub> (i = 4 to n) in the same manner as in (2) and (3), wherein positions A<sub>i</sub>-A<sub>n</sub> are substituted in any order; measuring enzyme activity of a sulfur atom-free enzyme protein obtained upon substitution of A<sub>n</sub>; and selecting a sulfur atom-free enzyme protein that retains activity.

13. (New) The process for producing a sulfur atom-free enzyme protein prepared by the stepwise mutation method of claim 12 wherein A<sub>1</sub>-A<sub>n</sub> are substituted in order of position on the amino acid sequence.

14. (New) A process for producing a sulfur atom-free enzyme protein using a combination of a combined mutation method and a stepwise mutation method, wherein the method comprises:

(1) preparing a mutant gene by substituting an initiation codon encoding L-methionine in a DNA sequence encoding an enzyme protein having an amino acid sequence comprising n number of sulfur atom-containing amino acids, wherein a position of a sulfur-containing amino acid to be substituted is designated as A<sub>i</sub> (i = 1 to k, k ≤ n), with codons encoding any of L-methionine-L-alanine, L-methionine-L-serine, or L-methionine-L-proline; expressing the mutant gene in a host cell to obtain a mutant enzyme protein; measuring enzyme activity

of the mutant enzyme protein; and selecting the protein with the highest activity, thereby obtaining a mutant enzyme protein having a substitution A1/MA1;

(2) preparing a mutant gene in which a codon encoding a sulfur-containing amino acid at a position  $A_i$  ( $i = 2$  to  $k$ ) is substituted with a codon encoding any of L-alanine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-glycine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-asparagine, L-proline, L-glutamine, L-arginine, L-serine, L-threonine, L-valine, L-tyrosine, and L-tryptophan, for a maximum of 18 different substitutions at any  $A_i$ ; expressing the mutant gene in a host cell to obtain a mutant enzyme protein; measuring enzyme activity of the mutant enzyme protein; and selecting a maximum of three mutant enzyme proteins having the highest activity, thereby obtaining mutant enzyme proteins having substitutions  $A_i/B_{i1}$ ,  $A_i/B_{i2}$ , and  $A_i/B_{i3}$ , wherein activity decreases in order  $A_i/B_{i1} > A_i/B_{i2} > A_i/B_{i3}$ ; and

(3) repeating (2) for all  $A_i$ ;

(4) producing a mutant enzyme protein comprising any combination of substitutions as in (1), (2), and (3), wherein a substitution occurs at all  $A_i$ ; measuring enzyme activity of the mutant enzyme protein; and selecting a maximum of three mutant enzyme proteins having the highest activity; and

(5) producing a sulfur atom-free enzyme protein by subjecting the mutant enzyme proteins selected in (4) to stepwise substitution of the remaining  $n - k$  number of sulfur-containing amino acids as in claim 12; measuring the enzyme activity of the sulfur atom-free enzyme protein; and selecting a sulfur atom-free enzyme protein that retains activity.